is sufficient to favour increased rapidity of cell-division and induce abnormal and varying forms.

4. Marine algæ grown in a limited volume of water and a limited supply of air in sunlight and full daylight fix both carbon and nitrogen rapidly into organic compounds. The amount of nitrogen fixed exceeds many times the total nitrogen originally present as ammonia, nitrite, or nitrate in the water. Moreover, the small initial amounts of nitrogen present in these forms are not decreased. It follows that the only available source is the free nitrogen of the atmosphere.

Studies on Synapsis. III.—The Nuclear Organisation of the Germ Cells in Libellula depressa.

By Lancelot Hogben.

(Communicated by Prof. E. W. MacBride, F.R.S. Received October 15, 1920.)

[Plates 4–7.]

An impressive array of facts in support of the chromosome hypothesis has been elucidated by the researches of cytologists during the past two decades; nevertheless the paucity of direct objective evidence for the integral continuity of the chromosomes through all stages of nuclear history still presents a barrier to unqualified acceptance of its validity. Such knowledge is not only essential to a convincing presentment of the chromosome hypothesis: it has furthermore a very intimate bearing upon the interpretation of synapsis.

The earlier workers, following Flemming, interpreted the prophase and telophase organisation of the nucleus as a continuous spireme: this notion rapidly hardened into a dogma, which not only handicapped further inquiry into the problem of the constitution of the resting nucleus, but proved to be a source of much confusion respecting the significance of the events in the meiotic phase. How far theory even succeeded in overriding fact may be illustrated sufficiently by a quotation from a paper that has exerted a powerful influence on cytological theory.* In reference to the spermatogonial prophases of Periplaneta, the authors state correctly, "the method of chromosome formation here depicted presents nothing exactly comparable to the long spireme thread which is figured in so many existing accounts of the

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* 'The Meiotic Phase in Animals and Plants.'

premeiotic divisions. . ." After this unequivocal statement the interpretation given to account for the reduction of the chromosomes is that "the spireme threadwork tends to separate out into half as many lengths" as the number of premeiotic chromosomes. Now, it may safely be said that in general (as well as in the case of Periplaneta) recent research has fully justified Bolles Lee in his contention that "ni à la télophase, ni aux prophases, ni à aucun moment de l'existence de ces noyaux, il y aît formation d'un spirème continu." Yet even recent workers, like Arnold, 1909 (1), Nakahara, 1919 and Harman, 1920 (2), in their inability to account for telosynaptic union by other means, fall back upon the conception of a spireme segmenting into the haploid number of threads as a means of effecting reduction.

The importance of an understanding of the various phases characteristic of nuclear division was emphasised in the last study in reference to comparisons made between the meiotic processes in Osmunda and Periplaneta. Attention was there directed chiefly to the question of whether the telophasic chromosomes divided in anticipation of the succeeding mitosis. A preliminary examination of spermatogenesis in Libellula in May, 1920, indicated that the material was of a very favourable kind for a more searching study of nuclear organisation in the premeiotic phase; and the present communication contains the results of observations made on material collected at that time and subsequently. The nymphs were identified, by the courtesy of Mr. H. Campion, of the British Museum, as *L. depressa.* Particulars of the materials and methods employed will be given with the observations recorded.

Previous Work on Odonata.

Since the Odonata, as a group, have attracted very little attention hitherto from cytologists, a brief summary of previous work may first be given. The only observations recorded with respect to the chromosome cycle are those of McGill, Lefevre, and Smith.

McGill, 1907 (3) has published an account of the oogenesis of two genera, Anax and Plathemis; the more salient particulars are: (*a*) the presence of yolk nuclei of the Lumbricus type in the young oocyte; (*b*) the condensation of the "synaptic spireme" around the plasmosome to form a double nucleolus; (*c*) the emission of fluid particles from the double nucleolus during the growth period of the egg. The author speculates that these latter are "reprecipitated" after "undergoing chemical change" to form the chromatin of the mature oocyte. The alleged condensation of the "synaptic spireme" around the plasmosome is homologised with synizesis in the male sex cells; and the views of Retzius, Leydig, Strassburger, Guignard, and Mertens, who regarded the nucleolus as a temporary form of chromatin storage, are believed to be reinforced by the study of the phenomena cited. Despite the unusual character of the process described and the interpretation inferred, no other investigator has repeated this work.

In 1908 Lefevre and McGill (4) repeated the earlier work of the latter on the spermatogenesis of *Anax junius*. In their amended form the chief points of interest recorded are as follows: (*a*) the diploid complex of the male germ cells in *A. junius* consists of twenty-seven chromosomes (including twelve pairs of rod-like, one accessory, and a pair of micro-chromosomes); (*b*) the chromosomes of the spermatogonial telophase form a continuous spireme; (*c*) the long axis of the tetrad is equivalent to the line of cleavage of the diplotene filament; (*d*) the X-element is segregated in the homotype mitosis.

More recently, Smith, 1916 (5) has made a careful study of the male sex cells in *Sympetrum semicinctum* and *Libellula basalis*. In both species the spermatogonial metaphase displays twenty-five equal chromosomes; the tetrads are stated to be formed like the rings of the Periplaneta type and to divide in the same way; the accessory is said to pass over undivided in the first reduction division of *L. basalis*, but in Sympetrium to behave as in Anax.

The Spermatogonia.

From the examination of gonads taken from spring and autumn nymphs, it appears that *L. depressa* does not complete its life cycle in less than two entire years. Most favourable material for the study of spermatogenesis is obtained from nymphs in the early spring immediately preceding the final moult. The testes then consist of cysts of spermatogonia undergoing mitotic changes and of spermatocytes; a remarkable feature of the species is the very short duration of the resting stage of the nucleus. In very young cysts, composed of only a dozen spermatogonia, the dimensions of the nuclei considerably exceed those of the nuclei of cysts of numerous cells; and, as the cells are in any case small, the former provide most suitable objects for observation. While it would appear that the divisions follow rapidly upon one another, a circumstance which facilitates observation, it is rarely found that the cells of any particular cyst differ to any marked extent in the phase of nuclear history which they display; this fact is again helpful to an intensive study of individual stages. The material employed was fixed in Flemming's reagent (either with or without acetic acid), diluted with two volumes of aq. dest. For staining, thionin, Gentian violet, and Haidenhein's iron-alum hæmatoxylin were used.

The history of the nucleus of the spermatogonial cells may be commenced most conveniently at the metaphase. As more than a hundred individuals were obtained for this purpose, an abundance of preparations, with all the chromosomes well separated in the equatorial plate, were available; in every case there were seen to be twenty-three stout, rod-like and slightly curved elements of approximately the same size. In spite of the fact that Smith gives the number twenty-five for the diploid complex of *L. basalis*, it is without the slightest hesitation that the former figure is stated as the correct count in the case of *L. depressa.* In many preparations (Plate 4, fig. 1) there are seen two (or sometimes one) small granules among the metaphase chromosomes; these occur also in the metaphase figures of follicle cells and oogonia (figs. 26–7); and it was at first an attractive supposition that these may be the vestigial representatives of the additional pair of chromosomes in *L. basalis*, especially in view of the fact that Lefevre and McGill describe a pair of *m*-chromosomes in *Anax junius.* This possibility was fully explored; and it was found that three such bodies were present in many cases in prophase nuclei of spermatogonia, while one, two, or even three were present, separated well apart in the first spermatocyte metaphase (fig. 22). It will be remembered that McGill originally could only find one "*m*-chromosome" in Anax; and the inconstant number of these granules, taken in conjunction with their failure to segregate in the reduction division, makes it very improbable that they can be justifiably interpreted as elements of the diploid complex in *L. depressa* at least.

An alternative proposition, that they constitute a group of Y-elements, is disposed of by comparison with the oogonial nuclei. In the prophase, these granules are first seen at the time when the plasmosome disappears; and there are considerable indications that they represent persistent remnants of the nucleolus passed over from one cell generation to another (fig. 10). One of the most interesting discoveries made with the aid of the new technique is that of Carleton, 1920 (7), who has shown that one or more intranucleolar elements capable of fission in mitosis pass over from one cell generation to another, and constitute the centre around which the new plasmosome is formed in the late telophase. In the light of this discovery, as well as in view of the facts mentioned, the last supposition appears to be the most acceptable.

As the chromosomes pass to the poles of the spindle, they are seen to become more curved; they also give clearer evidence of individuality in size; in the late anaphase (fig. 2) they become more sharply curved, and one or two pairs are seen to become attenuated into looped filaments. There is no evidence of cleavage either in this stage or in that which follows. When the nuclear membrane is defined, the chromosomes are seen to spin out into finely beaded loops, the extremities of which are polarised on the side of the nucleus remote from the centrosome (figs. 3, 4). This polarisation is inevitable if the arc-shaped chromosomes of the anaphase are to retain the same axis of symmetry with respect to the achromatic spindle during the process of attenuation. As the telophase filaments become more elongated, their arrangement becomes increasingly more difficult to follow (figs. 5, 6). The chromatic filaments, however, at all stages remain in contact with the nuclear membrane; whereas the plasmosome, which may appear early in the telophase, while the individual chromosomes are still identifiable as such, is always seen to be in the centre of the nucleus. There is thus no justification for regarding the plasmosome as in any way connected with chromatin organisation of the nucleus.

If the history of the prophase chromosomes is traced chronologically backwards, it is seen that their earliest precursors that can be definitely identified are in the form of attenuated, finely-beaded and convoluted filaments. As these become abbreviated, they show at first a definite orientation with respect to the plasmosome (figs. 7–10). At no stage either in prophase or in telophase is there any justification for regarding the individual filaments as united in a continuous spireme. The history of the chromosomes in the prophase recapitulates in obverse sequence the behaviour of their representatives in the preceding telophase. In this essential, the facts are in agreement with the conclusions drawn by Digby as regards Osmunda, 1919. But there is no evidence of cleavage for the ensuing metaphase, until the prophase chromosomes begin to assume the arc-shaped contour characteristic of the anaphase (figs. 9, 10). There is only one stage, of comparatively brief duration, in the germ nuclei of Libellula, when the individuality of the chromosomes is obscured; this is the so-called reticulate stage, or, as Bolles Lee has called it, the *spirophase;* and it will be noticed that there is no reason to infer that the individuality of the chromosomes is interrupted at this point. On the contrary, the phenomena described would more naturally lead to the conclusion that *the spirophase is in reality merely that point in the nuclear cycle, when the chromatin filaments become so elongated, and the chromomeres so widely separated that current methods of observation can no longer detect the direction of individual strands.*

The only recent account of the behaviour of the chromatin organisation of the nucleus during interkinesis on the Hexapoda is given by Schaffer, 1919 (8). In Lachnosterna (Coleoptera), " the chromosomes spin out into fine chromatic threads, and, as the nucleus grows, the threads become more and more complex . . . this resting nucleus is of relatively short duration, for soon the chromatin begins to condense into heavier threads." One very important resemblance between the events of the nuclear cycle in Libellula and Lachnosterna will be described later. While in general the few workers who have studied the interkinetic nucleus of late years agree that there is no loss of individuality in the "spirophase," the precise details of the process do not appear to be universally alike. In view of the importance attributed in Morgan's suggestive speculations to the linear alignment of the chromosome segments, it is tempting to seek for an interpretation of the spirophase in other forms along lines similar to those indicated above. But at present there is no justification for doing so.

There seem to be at least two distinct types of nuclear organisation in the resting stage. The first may be termed the *unit-reticula-complex;* in this case the telophasic chromosomes become increasingly vacuolated, until the spongy cylinders so formed cannot be distinguished, This at least is certainly the case in certain Orthoptera (*cf.* of Mohr, Buchner, et al.), where the individual reticula of the telophase chromosomes are separately invested in their own membranes; it is also the only possible interpretation of the diffuse stage in the oocyte.* The second type of resting nucleus is that to which the term spirophase was applied by Bolles Lee, 1912 (9), as the result of his very important work on *Paris quadrifolia.* With the exception of Dehorne, 1911 (10), workers of animal cells are agreed that there is no cleavage in the telophase threads; many botanical cytologists like Fraser, 1912 (11), and more recently Digby, adhere to the belief that the cleavage, which becomes manifest at one mitosis, begins in the anaphase or telophase of the preceding mitosis; but others, like Gates, 1912 (12) and Bolles Lee, find no evidence of preparation for the ensuing division until the prophase itself. One thing is evident in any case: the prodigious heterogeneity of structure of the chromosomes in the interkinesis as contrasted with their visible compactness of structure in the metaphase. And this should be borne in mind in reference to certain criticism which has been raised in reference to Morgan's hypothesis by Prof. Bateson in his recent Croonian Lecture.

The Meiotic Phase in the Male Germ Cells.

Lefevre and McGill do not give details respecting the initial processes during the synaptic period† in the primary spermatocytes of Anax. In the case of Sympetrum and *L. basalis*, Smith records the formation of leptotene filaments from irregular blocks of chromotin, as in Oncopeltus and Lygaeus (Wilson), following on a short reticulate phase. In no preparations of

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* Except perhaps in *Hymenoptera parasitica* (Hogben).

† American authors use the term "growth period" more conveniently applicable to the period when the oocyte grows in cytoplasmic bulk and yolk deposition occurs. "Maturation prophase" is better restricted for the formation of the definitive tetrads, especially in view of the telophasic significance of the earlier events. "Meiotic phase" includes both reduction divisions. Hence the use of a special term.

Libellula depressa was it possible to find any such stages. As already mentioned the telophase of the spermatogonial mitoses is characterised by an orientation of the filaments exactly like that which is found in the bouquet stage in Periplaneta; and in fig. 13 of Schaffer's paper (8) precisely the same occurrence is clearly illustrated. All the available evidence points to the conclusion that the *fine polarised leptotene loops of* Libellula depressa *display from the inception of the meiotic phase the arrangement characteristic of the chromatin filaments in the premeiotic telophase* (*cf.* figs. 4 and 11). As far as could be ascertained the leptotene filaments are from the first present as loops polarised at both extremities (fig. 11). It is very difficult to count the loops with accuracy; but their number is clearly more numerous than in the pachytene stage. In the contraction figure, they are drawn away from the nuclear membrane and become considerably abbreviated (fig. 13). Owing to their crowded disposition, it is not possible to observe with certainty actual pairing; but it is certain that throughout this stage and in that which follows, when it is possible to count with accuracy the haploid number of loops, they retain the original condition of polarisation at both extremities (figs. 13, 14).

Now if the polarisation of the leptotene loops is consequent upon the character of the last premeiotic telophase, there is no possibility in the case of Libellula that numerical reduction of the loops could be effected by means of an end-to-end union without an entire reorganisation of the chromatin organisation of the nucleus. In the absence of evidence for the intercalation of such a process, those who advocate telosynapsis for Hexapoda fall back on the conception of a continuous spireme; envisage reduction by the segmentation of the spireme into the haploid number of lengths: and declare themselves unable to find a previous (leptotene) stage in which the number of loops is more numerous. This contention is based on a fundamental misconception as to the nature of the telophase: when this is rightly appreciated, it follows that conjugation can only take place at one point in the sequence of meiotic phenomena—the bouquet contraction, and can only be effected by one means—the lateral approximation of the loops or filaments.

With respect to the diplotene stage (figs. 14, 15) in which the reduced filaments become extended and display longitudinal cleavage, it is only necessary to state that the nucleus as a whole undergoes considerable increase in bulk during this period (figs. 15, 16). Eventually the haploid number of longitudinally split filaments are detached (fig. 16); and their transformation into the definitive tetrads coincides with a progressive shrinking in size of the nucleus (figs. 17, 18).

The fate and constitution of the tetrads in the Odonata has provoked controversy. That they are formed by the opening out of the line of cleavage of the diplotene filaments is clear (fig. 17). But since some of the tetrads at the time when the nuclear membrane disappears, already exhibit the form of practically symmetrical crosses (fig. 18), there is difficulty in referring the plane of division to the axis of the filament from which they are derived. If the long axis of the tetrad when arranged on the achromatic spindle corresponds to the line of cleavage in the diplotene stage, the first division is reductive in the modern sense, and must separate univalents united end to end: otherwise it is reductive only in the sense in which Weismann interpreted meiotis, dividing each univalent transversely. In either case such a dispósition of the univalents in the metaphase of the heterotype mitosis would be incompatible with the theory of parasynapsis as now understood. This is the arrangement described by Lefevre and McGill for Anax; but it is clear from their figures that the sequence of the stages on which they base their contention is an inversion of the correct order. Smith adopts the alternative view in the case of Sympetrum and *L. basalis*; that is, the tetrad is so placed in metaphase that the transverse division of the tetrad involves the separation of the originally longitudinal halves of the diplotene segments. Though it is impossible to give objective demonstration of the correctness of this description for all the bivalents of *Libellula depressa*, at least two of them (*cf.* figs. 19 and 20) are symmetrical about one axis only, and show subterminal instead of median spindle fibre attachment. These are placed in the metaphase with their single axis of symmetry in the equatorial plane, and there is therefore no doubt whatever in regard to these that the heterotype division separates longitudinal halves of the diplotene bivalents. The similarity of these tetrads to the remainder in all other respects permits the inference that the latter behave in the same way.

In metaphase each bivalent is eventually draws out, as is Periplaneta, into an elliptical figure; and as each division completes itself the two arms of the dyads are drawn into closer contact. The homotype mitosis follows quickly on the telophase, and the chromosomes of the second division are actually not more than half the size of those in the first. Consequently it is not an easy task to follow out the prophase of the former, owing to the very small dimensions of the nuclei. A resting phase is not described by Lefevre and McGill in Anax, nor by Smith. But as far as *L. depressa* is concerned, there is such a stage, though of very short duration. The telophase chromosomes of the heterotype mitosis seem to spin out into loops as in the spermatogonia. The prophase chromosomes show cleavage lengthwise. Thus the homotype mitosis is equational, and cannot be referred to the transverse constriction of the dyad. This is exactly as described by Morse and the author in Periplaneta.

Throughout the foregoing account no mention has been made of the behaviour of the unpaired chomosome of the spermatogonia. As will become evident later, this chromosome represents the X-element or accessory of other forms. Throughout the entire synaptic period it can be identified as a club-shaped body (figs. 11, 15), becoming abbreviated in the metaphase I. In every case twelve chromosomes could be counted in the metaphase of the first heterotype division: usually they are arranged so that a central group of three is surrounded by a group of nine, including the accessory (fig. 22). In lateral views the X-chromosome can never be seen to pass to one pole undivided, but appears to undergo division rather later than the bivalents (fig. 21). Metaphase counts, however, of the homotype division display in some cases twelve, in other cases eleven chromosomes (figs. 23, 24). Their arrangement is generally the same as in heterotype metaphase, *i.e.*, an outer ring of nine (or eight) and an inner group of three. Lateral views indicate that the X-element passes to one pole undivided in the second reduction division of *Libellula depressa*, as in Sympetrum and Anax (fig. 25). Smith thinks, however, that this is not the case in *L. basalis* : it would nevertheless be remarkable if such a difference were confirmed in two species of the same genus.

It follows that in *Libellula depressa* the second division is reductional for the accessory chromosome. It has been stated by several writers of the parasynaptic school that where both divisions are longitudinal with respect to the diplotene filaments, it is impossible to identify one or the other as that in which the segregation of the autosomes is effected. But unless the pachytene filaments rotate about their long axes in the contraction phase, the line of cleavage in the diplotene stage must be in the same plane as that in which the conjugating elements approximate. It is, therefore, most reasonable in the absence of evidence to justify such a supposition to assume that the mitosis which separates the longitudinal halves of the diplotene segments is that in which the segregation of homologous chromosomes takes place. In general the pre-reductional view is the more satisfactory: but in the case of the Ascarid tetrad of which each joint corresponds to a longitudinal fissure of the bivalent filament, it is possible that either pre-reduction or post-reduction occurs. Possibly in the latter case the segregation of some of the bivalents occurs in one division and some in the alternate mitosis. The genetic ratios inferred would be the same.

Synapsis in the Oocyte.

Attention was originally attracted to a study of the gametogenesis of Libellula, on account of the remarkable phenomena described in connection with synapsis in the oocytes of two other genera of Odonata by McGill. Examination of the living ovaries showed that the nucleolus of the oocyte is relatively of enormous size; and it exhibits usually a double structure, having a central large vacuolar area; in addition, there is usually an extranucleolar body lying free in the nucleus. With a view to obtaining further data respecting the behaviour of the plasmosome in oogenesis, and with a desire to test the validity of McGill's interpretation of the similar double nucleolus of Anax and Plathemis,* the present research was begun; in the course of the work other points of interest emerged, as described above.

The point of signal interest in McGill's paper is the contention that the double nucleolus of the growing oocyte is formed by the condensation of the synaptic spireme (*i.e.*, bouquet) round the plasmosome. This process is homologised with synizesis in the male germ cells. The definitive chromatin organisation of the later egg is supposed by McGill to be formed by precipitation of substance secreted as globules by the nucleolus and dissolved in the karyolymph. The whole series of phenomena, it is maintained, reinforces the now generally discarded belief of Retzius, Leydig, Strassburger, and others, who regarded the plasmosome as a means of storing chromatin.

This is clearly at variance with (1) the general hypothesis of chromosome individuality, and (2) most accredited data bearing on the meiotic phenomena of oogenesis. It is usual to find in oogenesis, as in the case of Periplaneta, or Gryllus, that the diplotone filaments or pro-tetrads tend to become increasingly granular as the oocyte enters upon the phase of active growth in cytoplasmic bulk, so that typically the whole surface of the oocyte nucleus appears to become covered with uniformly distributed microsomes (chromatin granules) during the major part of the history of the egg. Eventually, the onset of the reverse process occurs, when the polar spindle is formed, *i.e.*, about the time the egg is laid or fertilisation occurs. In insects, with the exception pre-eminently of *Hymenoptera parasitica* (Hegner, Gatenby, Hogben), maturation does not begin in the ovarian egg. In a few cases, as in certain Amphibia, there is no "diffuse stage" at all (Jordan, King); the bivalents persist without such change throughout the entire growth of the oocyte. In yet other cases the bivalents become very diffuse, but are still individually recognisable, as in Pristiurus.

From these circumstances, and the fact that the chromatin units always reappear in the prophase, in the same manner and the same form as that in which they pass into the reticulate condition, it seems legitimate to infer, in the absence of evidence to the contrary, that there is no actual loss of individuality in the chromatin organisation of the oocyte. Evidence to the contrary has indeed been submitted and criticised in a previous contribution to this series, and further data bearing on this question will be considered

* The genus Plathemis has now been merged in Libellula.

later. The meiotic phenomena of *Libellula depressa* will be described first, and the question of the "chromidia" discussed in due course.

The ovaries in Libellula, as in Anax and Plathemis, are a pair of spindle-shaped capsules, enclosing numerous minute ovarioles. They are thus of the type found in Gryllus, consequently, they are not so amenable to very delicate fixation as are the freely-exposed egg tubes of Periplaneta. McGill's material was fixed whole—after dissection in fluid—in Flemming's reagent (strong formula). Comparison with preparations of Periplaneta showed that this technique is totally inadequate when attempted with the Libellula material. Improvement was obtained by adopting three precautions: first, the ovaries were removed without immersion in fluid and plunged directly in the reagent; secondly, they were teased out quickly and thoroughly, so that each ovariole was separately and instantaneously fixed; lastly, the fixing fluid (Flemming's) was diluted to a third the usual strength. When these precautions are observed, the results are in every way more satisfactory. *Mutatis mutandis* this applies to material preserved in chromosmic without acetic acid, and by the Mann-Kopsch method (*vide infra*) for the examination of mitochondria.

The ovarioles of Libellula, Anax, and Plathemis are of the simple type found in Gryllus and Periplaneta: the greater proportion consists of oocytes, progressively arranged with respect to size in linear series. The remainder consists of young oocytes at the inception of growth, lined side by side, a mass of oogonia and oocytes in synaptic stages, and a terminal filament of smaller cells. There are no nurse cells: the follicle cells arise from the primary oogonia and divide mitotically. A few authors have described amitotic division in follicle cells of Hexapoda, but in the majority of cases, probably all cases in which the follicle cells are germinal in origin, they divide in the usual way.

To obtain mitotic stages of oogonia and oocytes in synapsis, the second-year nymphs provide most suitable objects for study; for observations on yolk formation, the third-year nymphs, immediately before emerging, must be obtained. Oogonial mitoses are rare, and the chromosomes of the follicle cells are small and crowded. Hence it follows that the female germ cells do not provide suitable material for investigating the phenomena of nuclear division. A limited number of very clear oogonial anaphase, telophase, and prophase figures were obtained in the preparations studied, and in the main the series of phenomena seems to be essentially identical with what occurs in the spermatogonia. That is, in anaphase the chromosomes become more bent as they pass undivided to the poles of the spindle; in telophase they spin out into loops that are apparently polarised; in prophase the individual chromosomes are first recognised as attenuated filaments, progressively shortening into stout rod-like elements and undergoing cleavage simultaneously. The same is true of the follicular nucleus: in prophase it is usually seen that the separate chromosomes display appreciable differences of size *inter se.*

A few equatorial sections of nuclei in metaphase, both from follicle cells and oogonia, showed the chromosomes sufficiently widely separated to permit an accurate count of the diploid complex. There are in such cases twenty-four univalents: the unpaired element of the male germ cells is accompanied by a homologous partner (fig. 17). Minute deeply staining granules are seen among the chromosomes; but it does not appear that their number is constant, and there are suggestions that they are derived from the nucleolus in the prophase (fig. 26). The evidence in *Libellula depressa* is against the existence of a pair of m-chromosomes like those described by Lefevre and McGill in Anax.

At the inception of the meiotic phase the chromatin organisation of the nucleus consists of finely beaded, delicate, polarised loops in contact with nuclear membrane along their entire length. This condition is of comparatively long duration in the case of the oocyte, since it is found with greater frequency than other stages. Even in good sections it is difficult to count the number of loops with great accuracy; but by the study of sufficiently thin sections (3 μ) it is certain, that approximately, the full diploid number of filaments is present. Thus it corresponds to the leptotene stage of Von Winiwarter. It is illustrated in fig. 28. A plasmosome is always seen—usually towards the polar side of the nucleus where the leptotene filaments converge. There is no striking difference among the latter which would lead to the identification of the accessories in the early meiotic stages. As in so many other cases where the male X-element is conspicuously recognisable in synapsis, the accessories of the female germ cells behave in all respects like the autosomes. The only qualification which this statement merits is that in the metaphase of follicular mitoses two chromosomes are distinctly larger than their fellows.

The bouquet figure now passes through the stages of contraction seen in Periplaneta: the leptotene loops are withdrawn from the nuclear membrane, become shorter and thicker, and are in consequence more closely approximated side by side. There is one noticeable difference between the two cases; in Libellula, as in Tomopteris, the process is not synchronous for all the elements concerned; hence, while it is difficult to state dogmatically that there is a parallel conjugation in pairs of all the loops, it is possible to affirm without any shadow of doubt that such is the case with those which lag behind the rest (fig. 29).

In such cases it can be seen, as might be anticipated from the orientation of the loops, that approximation begins at the polarised extremities of the filaments extending distally till there is only a small portion where they still remain apart. The thickening of the filaments and their reduction numerically, as will be seen later, is effected in this process, and a consideration of the disposition of the loops makes it evident that reduction could only be brought about by parasyndesis. Though the loops, now bivalent, contract considerably towards the polar side of the nucleus with plasmosome to the side of or in the centre of the bouquet figure (fig. 30), in really good preparations there is nothing strictly comparable to the compact synizesis of some authors. A comparison between what occurs in Libellula and McGill's figures of Anax and Plathemis will suffice to make clear that what is described as the condensation of the synaptic spireme round the nucleolus in these two genera corresponds to maximal contraction in Libellula (fig. 30). Indeed, precisely similar appearances to those described by McGill are seen in preparations of Libellula made originally by the technical methods employed by that investigator.

The chief hiatus in McGill's account of the meiotic phase of the oocyte in Anax and Plathemis concerns the subsequent events. And there can now be little doubt that this is due to the short duration of the post-synaptic stage which intervene before the diffuse condition of the oocyte nucleus. It is a curious fact that, whereas in probably the majority of animals, the diplotene stage is that of longest and leptotene of shortest duration in the series of meiotic phenomena, the immediately post-synaptic stages in the oocytes of Libellula are extremely rare compared with the earlier ones. Thus a liberal supply of nymphs must be available if the complete history is required. And here opportunity arises to reiterate the paramount necessity of examining very large quantities of material for elucidating the whole sequence of events in the nuclear history of the germ cells: probably there is no more common occasion for error that has arisen in connection with the work which has been published in relation to the controversies which centre round the interpretation of synapsis than the extreme rapidity with which in certain cases some of the most critical stages are passed. For this reason negative evidence such as the failure of Nakahara and Arnold to find typical leptotene stages cannot carry the conviction which its exponents appear to anticipate.

After remaining for some time in maximal contraction, the pachytene loops become sufficiently loosened to permit exact numerical estimation (fig. 31) in sections at right angles to the axis of the bouquet: it is then seen that only the haploid number are now present. Numerical reduction is thus effected with certainty in the contraction process. As the pachytene loops extend into contact with the nuclear membrane (fig. 32), the longitudinal cleavage of the diplotene stage becomes evident. There follows the dissolution of the bouquet; and finally the abbreviation of the diplotene loops into segments of which the longitudinal halves become drawn apart except at their extremities (fig. 33). These latter condense to form tetrad-like bodies, which soon become increasingly granular in appearance, so that individually they are no longer recognisable, the surface of the nucleus in good preparations being apparently evenly covered with chromatin granules. Thus the diffuse stage supervenes in Libellula as in Periplaneta; and throughout the entire series of events the plasmosome remains altogether independent of the chromatinic organisation of the nucleus (fig. 35). Thus it is necessary to seek for some alternative interpretation of the double nucleolus of *Libellula depressa* to that offered by McGill for Anax and Plathemis; and when, the subsequent history of the oocyte nucleus in Libellula has been stated, there will be found little cause to doubt that the same procedure is in reality to be found in the other two genera.

Before passing on to an examination of the genesis and significance of the double nucleolus, it is interesting to compare the chromosome cycle of the Odonata with that of other Hexapoda. The early differentiation of the tetrads and the retarded segregation of the X-element in the male germ cells is similar to what has been described by Wilson for several genera of the Hemiptera. On the other hand, the definite polarisation in the synaptic stage conforms to the Orthopteran type. In Libellula, as in Periplaneta and Gryllus, the initial meiotic stages are of longer duration in the female germ cells than in the spermatocytes; also, owing to the larger size of the nuclei, the former are more favourable for investigation; it is therefore important, in the interpretation of synapsis, that work on the gametogenesis of both sexes should go hand in hand. It is true that, as the parasynaptic view has gained in prestige, some authors, *e.g.*, King (13), have pressed the compromise suggested by Gates (12) to the point of advocating both metasyndetic and parasyndetic reduction not only in closely allied species, but in alternate sexes of the same species. This position may satisfy those whose attention has been focussed exclusively on the frequently very intricate task of elucidating the complicated metamorphosis of the spindle bivalents, the conjugant halves of which in the final stage are often united only by one extremity. But, as Agar has rightly insisted, a survey of the entire sequence of changes reveals such striking similarity, more especially in the universally characteristic diplotene stage, that a satisfactory common ground is not easy to find for cytologists of the conflicting schools of opinion by any such facile means. Furthermore, as

Agar points out, in so many cases where earlier accounts (many Orthoptera, Platyhelminthes and Amphibia) appeared to support the telosynaptic interpretation, later examination of the very same animals provided evidence of parasynapsis.

Even accepting provisionally the possibility of pairing of both types in reduction among different animals, a difficulty emerges from the study of the premeiotic prophase and telophase, which to the author's knowledge no animal cytologist, who has of recent years advocated telosynapsis, has attempted to dispel. That many plant cytologists have succeeded in doing so is well known; and it has already been insisted in a previous paper that the similarities of the meiotic phase in animals and plants may have been grossly exaggerated. The possibility of relating the leptotene stage to the previous telophase in animals emphasises the dissimilarity further: that is, if actual synapsis in plants accompanies second contraction. *Since modern research has failed to find any confirmation for Flemming's continuous spireme, the task devolves on those who adhere to terminal as opposed to lateral conjugation in animals to state (in terms consonant with the known behaviour of the chromosomes in the meiotic and premeiotic nucleus) at what precise point this process is supposed to take place.*

The So-called Double Nucleolus.

Though the belief in the individuality of the chromosomes as persistent units of cell structure increasingly finds favour among those who study the problems of the cell, it is idle to overlook the fact that many current observations are clearly antagonistic to it. One such has been dealt with in the previous paragraph; a second, the question of the "chromidia" and so-called secondary nuclei has been touched upon in previous studies (6). On this point the behaviour of the plasmosome in the oogenesis of Libellula (figs. 36-39) is specially instructive; and a brief consideration of it will make clear how McGill was misled in describing the origin of the double nucleolus of Anax.

A reference to Jorgensen's work (14) will amply illustrate the capricious changes in staining capacity of the plasmosome accompanying the growth of the oocyte. Most commonly the nucleus of growing oocytes in preparations of Libellula fixed with Flemming's reagent and stained with Auerbach's method, exhibit a nucleolus consisting of a large medullary portion which is fuchsinophil and a surrounding cortex stained with methyl green. Precisely the same type of nucleolus is described by McGill, who failing to find typical stages succeeding contraction and led astray by the belief that fuchsin and methyl green are an infallible means of differentiating chromatin from other cell constituents, concluded that the double nucleolus is formed by the condensation of the synaptic spireme around the plasmosome during maximal contraction. But complicated methods of differential staining cannot suffice to shed light on cell problems, unless proper attention is paid concurrently to the technique of fixation; and McGill's materials were entirely fixed in fluids containing acetic acid. As a matter of fact, the plasmosome is uniformly fuchsinophil after fixation with Flemming's modified formula (Gatenby), *i.e.*, unless rendered basophil by previous treatment with acetic acid; this is true of all stages in oogenesis, both for Libellula and Periplaneta. There is no need to recapitulate previous comment (6*b*, pp. 315–319) on the value of differential staining; the conclusions built up within the scaffolding of the now discarded binuclearity hypothesis indicate the sources of error that arise from data derived in this way only.

Having examined living material in Libellula, it is possible to state with greater confidence what occurs in the nucleus during the diffuse stage. At the inception of the period of growth, after fixation in Flemming's, Bouin's, or Tellyesnicsky's fluids, the plasmosome, which undergoes at this time rapid increase in size, is seen to have changed its staining reaction. This may merely be a physical consequence of increase in surface (*cf.* Fischer's granules); and does not necessarily denote alteration of chemical constitution. Intranucleolar vacuoles make their appearance and increase in volume *one at a time*, each one successively enlarging, until it occupies the greater part of the plasmosome, while simultaneously becoming oxyphil in its reaction. *Thus the apparent double nature of the plasmosome results not from the absorption of any part of the chromatinic organisation of the nucleus, but from a process of internal differentiation.*

The only difference between the behaviour of the plasmosome in the oocytes of Libellula and Periplaneta in this respect is that the vacuolar bodies within the nucleolus attain their full size singly in the former, whereas several mature at a time in the latter. Their subsequent fate is similar in both genera. Each of these vacuolar bodies, which for ulterior reasons as well as to avoid the confusion between chromatin, mitochondrial, and nucleolar substance suggested by the older term "chromidia" have been designated deutosomes (6), emerge from the plasmosome into the karyolymph. According to McGill in the case of Anax and Plathemis they dissolve in the karyolymph; but in preparations made with the mitochondrial technique of Gatenby and Kopsch they are found to pass out into the cytoplasm to the periphery of the egg, where they eventually break up into smaller spheres which become the first vitellus (fig. 39).

Since the nuclear origin of the mitochondria has been discredited, cytologists have been very hesitant to admit the passage of material from the nucleus to the cytoplasm; partisans of the mitochondrial and chromosome hypotheses have been equally eager to disclaim any such suggestion. That the plasmosome plays an important metabolic *rôle* in oogenesis, and in a number of cases actually secretes fluid or solid particles that pass as such into the cytoplasm is, however, clearly indicated by the recent work of Hempelmann (15) and Buchner (16) on the *Archiannelid Saccocirrus*, Dendy on *Grantia*, and the testimony of numerous writers who have lately examined the secondary nuclei of the Hexapod egg. While some confusion arises by the use of the term "chromatin" (17) for such particles, since structurally the plasmosome and chromatin are separate and no valid chemical tests indicate their identity, it now seems possible to place the whole question of the transmission of nuclear material to the cytoplasm with the genesis of the secondary nuclei upon a basis which is not incompatible with what is generally accepted regarding the behaviour of chromatin and mitochondria in the germ cycle.*

Buchner, in particular, traces the origin of secondary nuclei in the Hymenoptera to the plasmosome; and the author has recently examined preparations of the ovaries of Crœsus, one of the Tenthredinidæ (unpublished), in which this is certainly the case.† Now if the plasmosome is to be added to the list of independent cell organs, as Carleton's work in itself strongly indicates, it may seem at first highly improbable that bodies so closely simulating true nuclei as do the accessory nuclei of the Hymenoptera should not be chromatinic in origin. Though characteristic secondary nuclei are not found in Libellula, the behaviour of the plasmosome in this form, as also in Anax and Plathemis, is here of great interest. It will be remembered that the intranucleolar deutosomes of Periplaneta were said to have the appearance of minute nuclei. In the nucleolus of Libellula, the oxyphil medulla (intranucleolar deutosome) is at first of finely granular structure in fixed preparations. This is indicated in figs. 36, 37, but in illustration its extraordinary resemblance to a nucleus cannot be reproduced in such a way as to do justice to the original. In short, *the oogenesis of Libellula shows that bodies may be formed within the plasmosome itself which have as genuine a resemblance to true nuclei as the secondary "nuclei" of the Hymenopteran egg: this reinforces the evidence for regarding the latter as a product of the plasmosome; and the undoubted emission of nuclear material during oogenesis no longer necessitates*

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* Cytomixis in Œnothera (Gates) merits re-investigation from this standpoint.

† By the courtesy of Dr. Gatenby I have recently seen preparations of Apanteles which show vacuolation of the plasmosome and presence of accessory nuclei in the diplotene stage; this leaves no doubt, in my mind, that the latter are nucleolar rather than chromatinic in origin.

the view that the integral continuity of the chromatinic organisation of the nucleus is interrupted in the diffuse stage.

The Yolk Nucleus of Libellula.

In concluding this record of the history of the ovarian oocyte in *L. depressa* reference is due to a crescentic body found in a juxtanuclear position in Anax; this is stated to undergo fragmentation and dispersal in the cytoplasm of the egg as growth proceeds. McGill calls it the yolk nucleus on the analogy of a similar structure described by Calkins and Foot in the egg of Lumbricus and Allobophora. Such a body is also present in the oocytes of *L. depressa.* After the examination of preparations fixed in Flemming's modified formula (without acetic acid), and by the Mann-Kopsch method,* followed by turpentine to remove osmicated fat, it appeared that the yolk nucleus is in reality the mitosome or chondriome of the oocyte, being the area in which the mitochondria are congregated. This not infrequently resists injurious fixation in young oocytes—perhaps because the protein component still differentiates from the ground cytoplasm after the lipins have been extracted. Gatenby has described the dispersal of the mitochondria in the oogenesis of Apanteles recently; and the facts are essentially similar in Libellula, except that in the latter the mitochondria do not appear to become filamentous. An interesting point noticed was that in some cases the spherical bodies emitted from the plasmosome and lying in the periphery of the egg outside the mitochondrial area were sometimes seen to be invested with a cloud of mitochondrial granules (figs. 38, 39).

The work of Gatenby (17), Hirschler (18) and others has shown quite clearly that both mitochondria and dictyosomes (Golgi rods) may be transformed into deutoplasmic spheres. On the other hand, Gatenby and Nusbaum Hilarowicz (19), who has recently demonstrated a Golgi apparatus in Dytiscus eggs, were not able to trace the origin of the yolk in the Hexapod egg to the cytoplasmic inclusions. Is it therefore necessary to conclude that the mechanism of yolk formation with respect to the cell organs is totally different in different animals? The observation recorded above seems to offer an alternative. Jorgensen's researches, though unfortunately largely vitiated by inadequate technique, do at least show that yolk formation throughout the animal kingdom coincides with a period of intense metabolic activity on the part of the plasmosome; it is equally certain that the process of yolk formation in general corresponds with a period of rapid increase in number of the extra-nuclear cell elements. Is it not therefore more reasonable to accept as a provisional hypothesis the view that yolk formation is a very intricate interaction of the metabolic functions of plasmosome, mitochondria and Golgi apparatus, a hypothesis which, while unifying the data, is perfectly compatible with the observed transformation of deutosomes (nuclear emissions), chondriosomes and dictyosomes in individual cases? It is in no way implied that Schaxel's account of nuclear emission in the vitellogenesis of hydroids is accepted; for a criticism of this work, Beckwith's paper (20) may be consulted.

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* Saline corrosive 2½ hours, aq. dest., osmic acid 2 per cent. 10 days.

Synopsis.

(1) Nuclear organisation in the germ cells of *L. depressa* has been investigated; emphasis is laid especially on two points: (*a*) the importance of the study of premeiotic kinetic phases to the interpretation of synapsis; (*b*) the independence of the plasmosome as a nuclear constituent from the chromatinic organisation.

(2) The diploid complex in the male germ cells of *L. depressa* consists of twenty-three approximately equal chromosomes, that of the female consisting of twenty-four.

(3) The chromosomes of the premeiotic mitoses become more curved and display greater individuality *inter se* as they pass undivided to the poles of the spindle; in the telophase they spin out into finely granular loops which initially occupy a polar disposition; they become increasingly attenuated in the spirophase (reticulate stage), so that individually they cannot be identified: in the prophase the reverse process occurs; they are first recognisable individually as convoluted threads, contracting into beaded loops which become abbreviated to form longitudinally split arc-shaped chromosomes.

(4) The failure of modern cytological research to confirm the existence of Flemming's continuous spireme makes it imperative for those who advocate telosynapsis in animal forms to postulate some means whereby terminal union of homologous chromosomes can be effected in a manner consonant with the observed data respecting the premeiotic telophase and the synaptic processes. Zoological cytologists who have adopted this view have not yet offered such information.

(5) Parasynapsis can be observed in the oocytes of Libellula: the method conjunction is probably the same in the male germ cells.

(6) The transverse axis of the tetrad in the first spermatocyte mitosis corresponds with the longitudinal split in the diplotene stage; the X-element is segregated in the second reduction division.

(7) The behaviour of the double nucleolus in Libellula has been investi-

gated, and provides no sanction for the view that the plasmosome is in any way connected with the chromatinic organisation of the nucleus: its relation to yolk formation is indicated.

(8) Data relating to the transmission of material from nucleus to cytoplasm are discussed in their bearing on "chromidia" and secondary "nuclei"; it is submitted that the origin of the latter in no way involves a revision of the view that the chromatin organisation of the nucleus retains its integral continuity throughout the growth phase.

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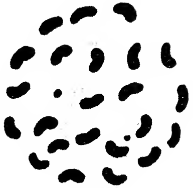
EXPLANATION OF PLATES.

Fig. 1.—Spermatogonial metaphase: 23 chromosomes and two granules ? nucleolar in origin. 2.—Do., anaphase. 3–5.—Telophase: *p*, plasmosome. 6.—Spirophase. 7–10. — Prophase. 11. — Spermatocyte: Leptotene stage, X, X-chromosome. 12.—Contraction phase. 13.—Do., pachytene. 14–15.—Diplotene stage. 16.—Dissolution of bouquet. 17–18.—Genesis of tetrads. 19–22.—Heterotype mitosis. 23–25.—Second reduction division with 11 and 12 chromosomes. 26.—Oogonial prophase. 27.—Metaphase with 24 chromosomes. 28.—Oocyte: leptotene stage. 29.—Zygonema. 30.—Maximal contraction. 31.—T.S., late pachytene stage. 32.—Diplotene stage (post-synaptic). 33–35.—Genesis of pro-tetrads and inception of the diffuse stage. 36.—Oocyte in growth period with double nucleolus (*p*), nucleus-like intranucleolar oxyphil body (*o*), chromatic network (*cr*), deutosomes in nucleus and cytoplasm (*d*). *Flemming.* 37.—Do., showing formation of the oxyphil body. *Flemming.* 38.—Oocyte at inception of growth period with yolk nucleus or mitosome. *Mann-Kopsch.* 39.—T.S. older egg, mitochondrial area (*m*). *F. W.A.*



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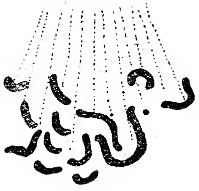

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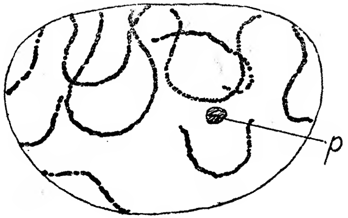

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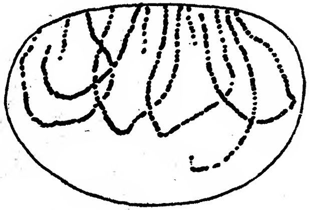

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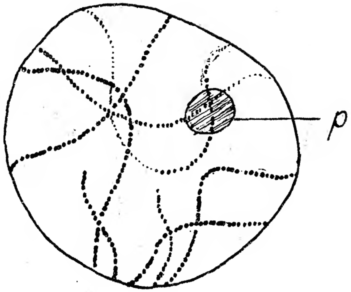

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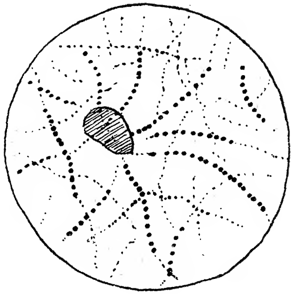

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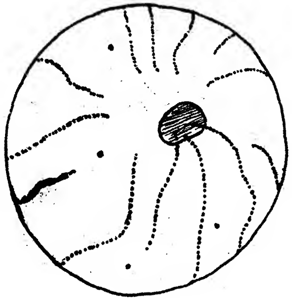

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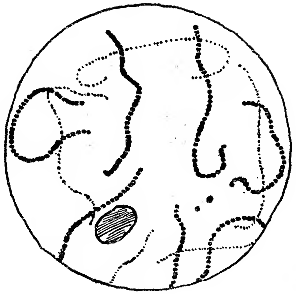

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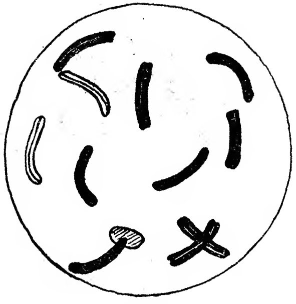

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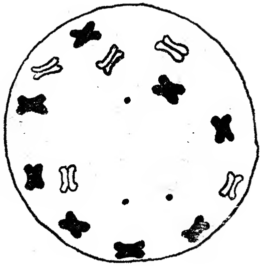

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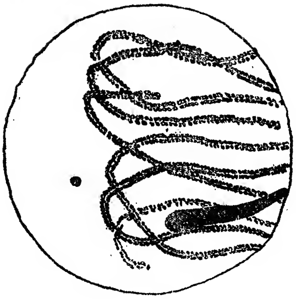

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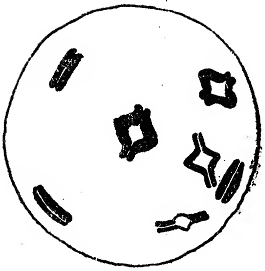

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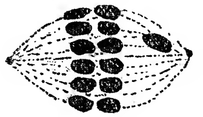

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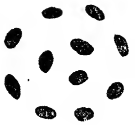

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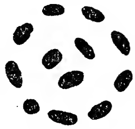

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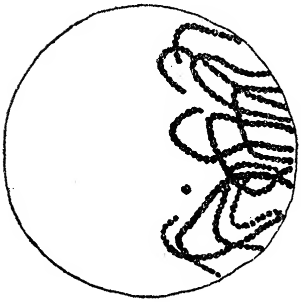

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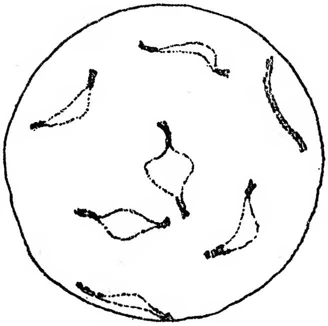

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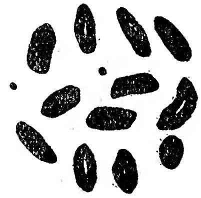

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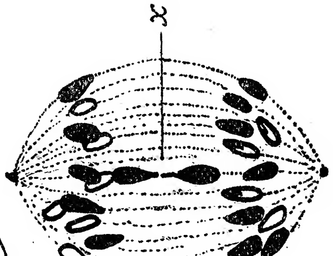

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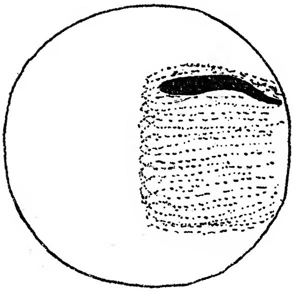

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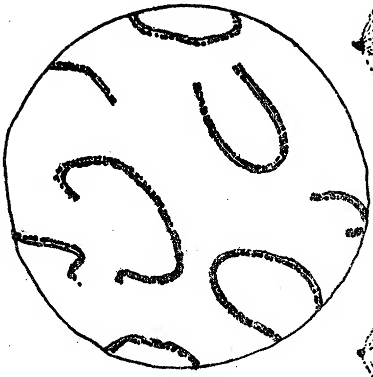

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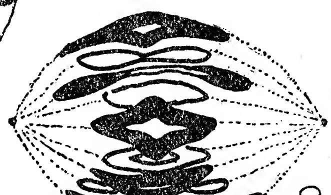

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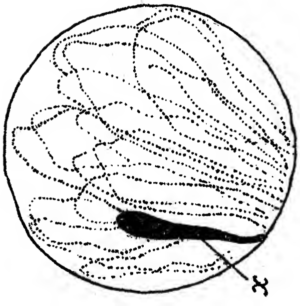

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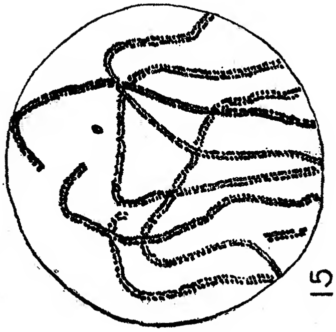

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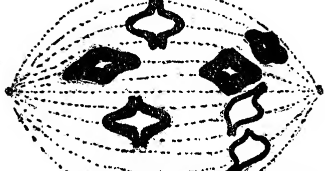

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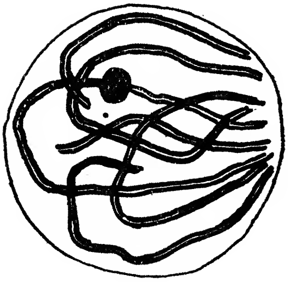

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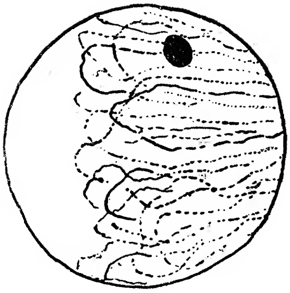

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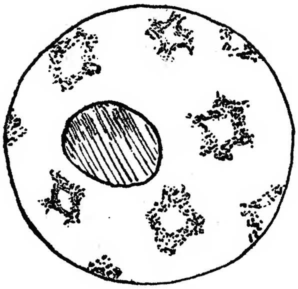

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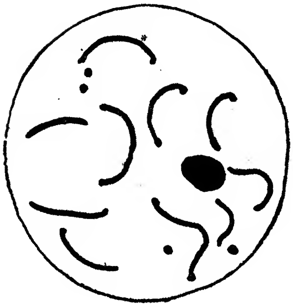

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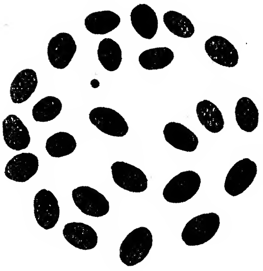

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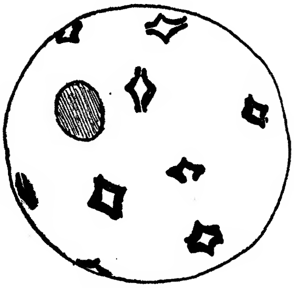

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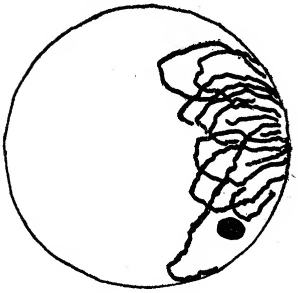

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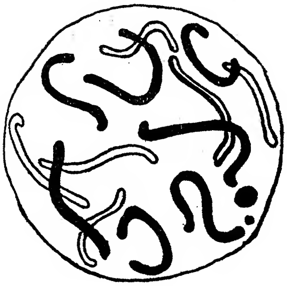

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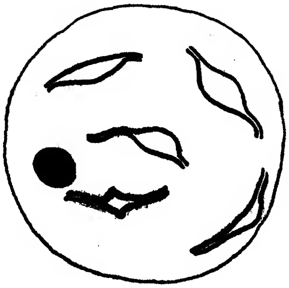

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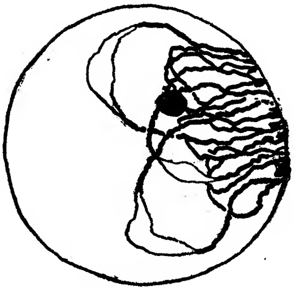

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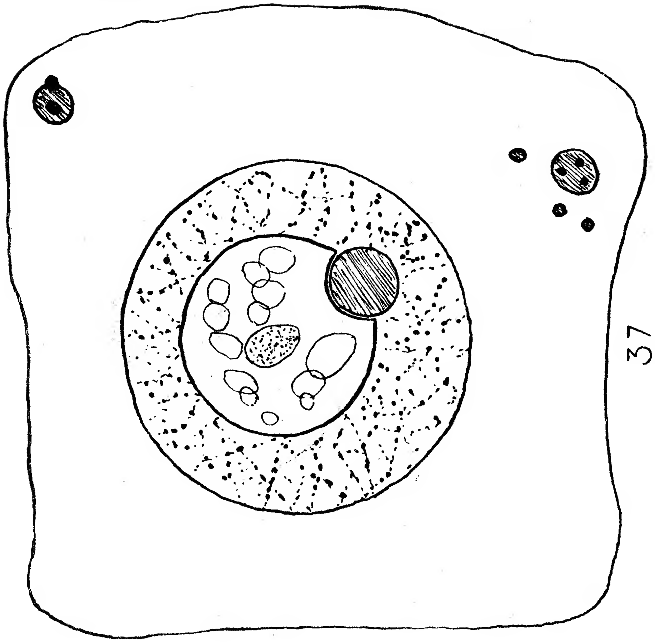

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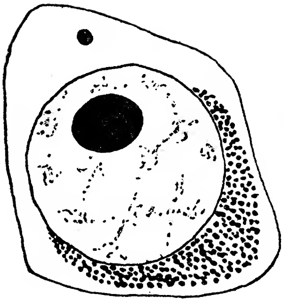

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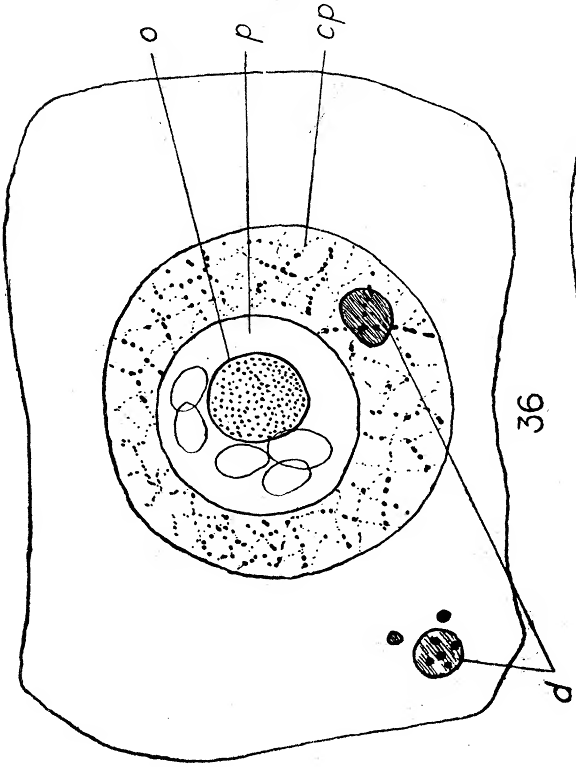

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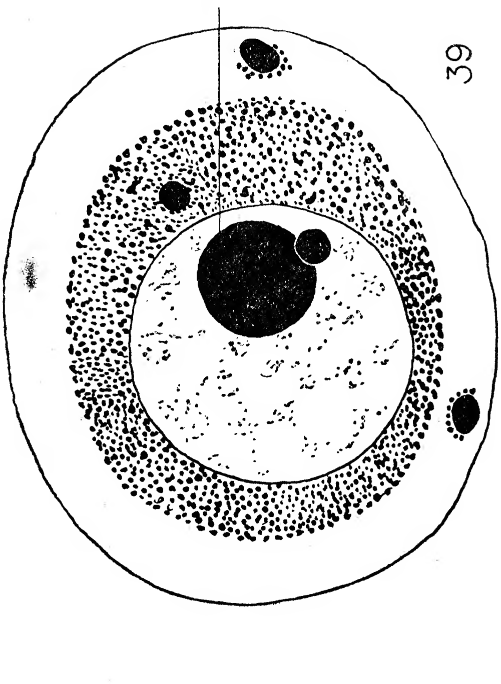

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